Original Article

Pathological Regression by Angiotensin II Type 1 Receptor Blockade in Patients with Mesangial Proliferative Glomerulonephritis

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Although angiotensin II type 1 receptor blocker (ARB) therapy reduces proteinuria and retards the progression of renal injury in patients with glomerulonephritis, whether these drugs actually ameliorate pathological damages in human glomerulonephritis has not been determined. Fifteen patients with biopsy-proven mild-to-moderate mesangial proliferative glomerulonephritis (10 with immunoglobulin A [IgA] nephropathy and 5 with non-IgA mesangial proliferative glomerulonephritis) received ARB monotherapy. In these patients, repeated renal biopsy was performed after a mean of 28.1 months, and pathological changes (including the mesangial matrix expansion ratio and interstitial fibrosis expansion ratio) were quantitatively examined using an image analyzer. Clinical markers were also evaluated, including the serum creatinine, serum IgA, creatinine clearance (Ccr), 24-h urinary protein excretion, urinary *N*-acetyl-*β*-D-glucosaminidase (NAG), and blood pressure. ARB therapy significantly reduced urinary protein excretion (0.68±0.63 to 0.20±0.32 g/day, $p=0.016$) and the blood pressure (systolic: 133.3±18.2 to 123.4±10.5 mmHg, $p=0.041$; diastolic: 79.4±11.9 to 72.0±8.2 mmHg, $p=0.038$). Although the global glomerular sclerosis ratio was unchanged (6.3±8.5% to 10.7±16.1%, $p=0.33$), the mesangial matrix expansion ratio (33.1±10.8% to 22.7±7.8%, $p=0.001$) and the interstitial fibrosis ratio (19.9±5.8% to 13.8±4.4%, $p=0.034$) were significantly reduced by ARB treatment. The levels of pathological improvement were similar between patients with IgA nephropathy and those with non-IgA mesangial proliferative glomerulonephritis. The results of the present study strongly suggest that ARB monotherapy can significantly reverse pathological changes, including mesangial matrix expansion and interstitial fibrosis, in human glomerulonephritis. (*Hypertens Res* 2008; 31: 387–394)

***Key Words*: mesangial proliferative glomerulonephritis, angiotensin type 1 receptor blocker, mesangial matrix, interstitial fibrosis, angiotensin II**

Introduction

Angiotensin II type 1 receptor blocker (ARB) therapy has a renoprotective effect in many experimental animal models, including partial nephrectomy models (*1–8*) and glomerulonephritis models (*9–11*). Although ARBs lower blood pressure, a renoprotective effect of ARBs has been demonstrated in both hypertensive and normotensive renal injuries, and is thought to be one of the "beyond blood pressure-lowering" effects. Thus, ARB therapy is one of the most promising ways to slow the progression of chronic renal damage.

Although experimental studies using animal models and/or cells have established the benefits of ARBs, the advantages of ARB therapy in the clinical setting are still being documented. ARBs reduce proteinuria and improve the clinical course in patients with immunoglobulin A (IgA) nephropathy (*12–14*), type 2 diabetic nephropathy (*15–18*), and focal seg-

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Table 1. Basic Characteristics of 15 Patients with Mesangial Proliferative Glomerulonephritis

	Total (n=15)	IgA-GN (n=10)	Non-IgA-GN (n=5)
Age (years)	51.3±13.1	53.0±13.5	48.0±12.7
Sex (male/female)	11/4	8/2	3/2
Urea nitrogen (mg/dL)	15.5±4.5	15.6±5.4	15.2±5.1
Creatinine (mg/dL)	0.92±0.23	0.96±0.20	0.81±0.31
Ccr (mL/min/1.73 m^2)	91.3±25.2	87.6±21.9	102.6±36.5
Serum IgA (mg/dL)	308.1±133.0	353.9±138.6	216.6±61.9[a]
Urinary protein excretion (g/day)	0.68±0.63	0.64±0.64	0.73±0.60
Grade of hematuria	0.97±0.92	0.95±0.83	1.00±1.20
Urinary NAG (U/L)	8.0±7.5	7.1±8.1	10.3±6.3
Blood pressure (mmHg)			
Systolic	133.2±18.2	137.8±19.1	119.0±2.6
Diastolic	79.4±11.9	79.0±13.1	80.5±10.6
Duration between two biopsy (months)	28.1±15.5	28.1±14.0	28.2±19.9
Angiotensin receptor blocker (n)			
Losartan	10	7	3
Valsartan	4	3	1
Telmisartan	1	0	1
Fasting blood glucose (mg/dL)	100.1±13.1	101.7±16.6	97.4±4.3
Total cholesterol (mg/dL)	197.5±26.7	196.6±27.1	199.7±29.8
HDL-cholesterol (mg/dL)	49.0±10.5	52.2±11.5	43.8±6.8
LDL-cholesterol (mg/dL)	111.8±24.7	115.4±23.8	104.7±28.5
Triglyceride (mg/dL)	148.9±53.4	152.4±22.1	140.7±23.0
Body mass index (kg/m^2)	22.9±1.9	22.3±1.7	24.5±1.3[a]

GN, glomerulonephritis; Ccr, creatinine clearance; IgA, immunoglobulin A; NAG, *N*-acetyl-β-D-glucosaminidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a]$p<0.05$ *vs*. IgA-GN.

mental glomerulosclerosis (*19*). However, it is unclear whether or not ARB therapy actually induces pathological improvement in patients with glomerulonephritis, as it does in experimental animal models of renal injury.

Accordingly, the purpose of this study was to verify the influence of ARB therapy on pathological changes in patients with mesangial proliferative glomerulonephritis along with its clinical effects.

Methods

Patients

A total of 289 patients underwent renal biopsy over 4 years from October 1999 to September 2003 at our renal division. Among these patients, 57 were diagnosed as having IgA nephropathy (IgA-GN) and 42 patients had non-IgA mesangial proliferative glomerulonephritis (non-IgA-GN). Non-IgA-GN was diagnosed when biopsy findings fulfilled the following criteria: 1) mesangial matrix and/or cell proliferation, and 2) no dominant IgA deposition in the mesangial area by immunofluorescent microscopy. Patients with systemic and/or secondary diseases such as systemic lupus nephritis with mesangial proliferation were excluded from this study population. Exclusion criteria for this study were as follows: pregnancy, malignancy, systolic blood pressure <100 mmHg, systolic blood pressure >180 mmHg that could not be controlled by ARB monotherapy, creatinine clearance (Ccr) <30 mL/min/1.73 m^2, and proteinuria >3 g/day. Thus, among the 99 patients with mesangial proliferative glomerulonephritis, 15 patients with mild-to-moderate mesangial matrix proliferation (10 patients with IgA-GN and 5 patients with non-IgA-GN) fulfilled the above criteria, and all 15 patients received ARB monotherapy after giving written informed consent. ARB was initiated in all 15 cases within 4 weeks after renal biopsy. ARB therapy consisted of losartan (100 mg/day) in 10 patients, valsartan (80 mg/day) in 4 patients, and telmisartan (40 mg/day) in 1 patient (Table 1). These ARB medications were not changed during the follow up period, and other therapy such as protein restriction and/or anti-platelet drug administration was not done during this study. All of the patients repeatedly underwent the second renal biopsy after a mean of 28.1±15.5 months to evaluate pathological changes (including the glomerular sclerosis ratio, mesangial matrix proliferation ratio, and interstitial fibrosis ratio) along with evaluation of their clinical characteristics.

Laboratory and Clinical Parameters

The laboratory and clinical parameters examined at the time of the first and second renal biopsy included the serum creatinine (mg/dL), serum IgA (mg/dL), grade of hematuria, 24-h urinary protein excretion (g/day), urinary *N*-acetyl-β-D-glucosaminidase (NAG) excretion (U/L), Ccr (mL/min/1.73 m^2), and blood pressure (mmHg). Ccr was calculated from 24-h urinary creatinine excretion and serum creatinine level. Hematuria was scored as negative, 0; ±, 0.5; +, 1; ++, 2; and +++, 3.

Histological Evaluation

Renal biopsy was done under ultrasonographic guidance using a Bard® Monopty® biopsy instrument (Medicon Co., Ltd., Osaka, Japan). The 18-gauge biopsy needle had a 17 mm notch for tissue sampling at the top of the needle, and puncture of the kidney was usually done three times. Thus, three cores of renal tissue specimen were obtained in each patient. Renal biopsy specimens were fixed in 10% paraformaldehyde and embedded in paraffin. Then sections (2 μm thick) were cut and stained with periodic-acid-Schiff (PAS) stain and Masson's trichrome stain for morphological assessment. The number of glomeruli on light microscopy in each patient ranged from 15 to 26, thus enabling a sufficient evaluation for histological changes of glomeruli and tubulointerstitium. The histological control was provided by renal biopsy specimens from 4 age-matched patients (3 men and 1 woman, mean age of 46.7±7.8 years) with minor glomerular abnormalities. Histological evaluation was done by two independent nephrologists who did not have any information about the patients. The scores of the global glomerular sclerosis index, mesangial matrix expansion ratio, and interstitial fibrosis ratio, before and after the ARB treatment in each patient, were calculated as the means of the values obtained by two nephrologists.

Global Sclerosis Index

The number of glomeruli with global sclerosis was counted on a PAS-stained section and divided by the total number of glomeruli to calculate the glomerular sclerosis ratio (%).

Mesangial Matrix Expansion Ratio

An image analyzer (Image-Pro® Plus software; Media Cybernetics, Silver Spring, USA) was used for evaluation of the mesangial matrix area and glomerular tuft area. The PAS-positive mesangial matrix area was calculated at a magnification of ×200. Mesangial matrix expansion was expressed by calculating the ratio of the mesangial matrix area to the glomerular tuft area. Briefly, a photograph of a PAS-stained glomerulus was viewed on a computer display, and the "area ratio" was selected from the "measure" menu. Then the PAS-positive mesangial matrix area was selected. The "area of interest" was drawn freely along the capillary walls of the glomerular tuft, after which the mesangial matrix area/glomerular tuft area ratio could be calculated. In each patient, all of the glomeruli without global sclerosis were examined, and the mean mesangial matrix expansion ratio (%) was calculated.

Interstitial Fibrosis Expansion Ratio

The interstitial fibrosis area was also evaluated by image analysis. Using Masson-trichrome–stained sections of biopsy specimens, the blue-stained area of interstitial fibrosis was calculated at a magnification of ×100 by the method described above. Then the interstitial fibrosis expansion ratio was calculated as the interstitial fibrosis area divided by the tubulointerstitial area (the glomeruli and large vessels were excluded from the tubulointerstitial area). Five renal cortical areas were randomly selected in each patient and the mean interstitial fibrosis expansion ratio (%) was calculated.

Statistical Analysis

Results are presented as the means±SD. The group mean values were compared by two-tailed Wilcoxon *t*-test or Mann-Whitney *U*-test. Stat View 5.0 Software for Windows (SAS Institute, Cary, USA) was employed for data analysis on a personal computer, and values of $p<0.05$ were considered statistically significant.

Results

Patients

The clinical characteristics of the patients at the time of enrollment in the study are summarized in Table 1. There were 11 men and 4 women with a mean age of 51.3±13.1 years. Serum creatinine was 0.92±0.23 mg/dL (range: 0.7–1.3 mg/dL) and Ccr was 91.3±25.2 mL/min/1.73 m^2 (range: 56.5–114.7 mL/min/1.73 m^2). Twenty-four hour urinary protein excretion was 0.68±0.63 g/day (range: 0.3–2.3 g/day). The serum IgA level for all patients was 308.1±133.0 mg/dL (range: 124–640 mg/dL). The serum IgA level of IgA-GN patients was significantly higher than that of non–IgA-GN patients (353.9±138.6 *vs*. 216.6±61.9 mg/dL) at the start of the study.

Effects of ARB Treatment

As shown in Table 2, overall urinary protein excretion was significantly reduced by ARB treatment (from 0.68±0.63 to 0.20±0.32 g/day, $p=0.016$). ARB therapy also significantly lowered the systolic blood pressure (from 133.3±18.2 to 123.4±10.5 mmHg, $p=0.041$) and the diastolic blood pressure (from 79.4±11.9 to 72.0±8.2 mmHg, $p=0.038$). The improvements of urinary protein excretion and blood pressure were equally observed in IgA-GN and non–IgA-GN patients. Although urea nitrogen showed a significant increase from 15.6±5.4 to 17.7±5.2 mg/dL ($p<0.05$) in IgA-GN patients,

Table 2. Laboratory and Clinical Change by ARB Treatment

		Total (n=15)	IgA-GN (n=10)	Non-IgA-GN (n=5)
Urea nitrogen (mg/dL)	Pre	15.5±4.5	15.6±5.4	15.2±5.1
	Post	17.1±5.5	17.7±5.2[a]	15.7±6.4
Creatinine (mg/dL)	Pre	0.92±0.23	0.96±0.20	0.81±0.31
	Post	0.92±0.21	0.95±0.20	0.82±0.26
Ccr (mL/min/1.73 m^2)	Pre	91.3±25.2	87.6±21.9	102.6±36.5
	Post	96.8±27.5	92.2±25.7	110.4±33.7
Serum IgA (mg/dL)	Pre	308.1±133.0	353.9±138.6	216.6±61.9
	Post	299.0±113.3	332.7±129.7	238.4±31.3
Urinary protein (g/day)	Pre	0.68±0.63	0.64±0.64	0.73±0.60
	Post	0.20±0.32[a]	0.21±0.33[a]	0.17±0.30[a]
Grade of hematuria	Pre	0.97±0.92	0.95±0.83	1.00±1.20
	Post	0.50±0.63	0.55±0.50	0.40±0.90
Urinary NAG (U/L)	Pre	8.0±7.5	7.1±8.1	10.3±6.3
	Post	5.4±5.5	6.4±6.2	2.7±1.0
Blood pressure (mmHg)				
Systolic	Pre	133.3±18.2	137.8±19.1	119.0±2.6
	Post	123.4±10.5[a]	125.9±9.6[a]	113.3±10.6
Diastolic	Pre	79.4±11.9	79.0±13.1	80.5±10.6
	Post	72.0±8.2[a]	74.4±8.4	66.5±4.4

ARB, angiotensin II type 1 receptor blocker; IgA, immunoglobulin A; GN, glomerulonephritis; Ccr, creatinine clearance; NAG, *N*-acetyl-β-D-glucosaminidase. [a]$p<0.05$ *vs*. pretreatment value.

Table 3. Pathological Change by ARB Treatment

		Total (n=15)	IgA-GN (n=10)	Non-IgA-GN (n=5)	Control (n=4)
Mesangial proliferation ratio (%)	Pre	33.1±10.8[b]	32.8±9.3[b]	33.8±14.4[b]	11.6±2.8
	Post	22.7±7.8[b,d]	23.7±8.3[b,c]	19.2±5.9[b,c]	
Interstitial fibrosis ratio (%)	Pre	19.9±5.8[b]	20.5±5.8[b]	18.6±6.2[b]	5.4±1.7
	Post	13.8±4.4[b,d]	15.5±3.5[b,c]	10.5±4.3[a,c]	
Glomerular sclerosis ratio (%)	Pre	6.3±8.5	8.1±9.3	2.6±5.8	1.4±3.1
	Post	10.7±16.1	9.6±13.8	12.9±21.7	

ARB, angiotensin II type 1 receptor blocker; IgA, immunoglobulin A; GN, glomerulonephritis. Control specimen was obtained from age-matched patients with minor glomerular abnormality. [a]$p<0.05$, [b]$p<0.01$ *vs*. control value. [c]$p<0.05$, [d]$p<0.01$ *vs*. pretreatment value.

the overall urea nitrogen level was not significantly increased. Serum creatinine, IgA, Ccr, urinary NAG, and hematuria did not change significantly with ARB treatment. Estimated mean daily salt intake calculated by 24-h urinary sodium excretions was 8.9±0.9 g/day before ARB treatment, and 8.6±0.3 g/day by time-averaged monthly 24-h urinary sodium excretions during the ARB treatment in our subjects, and there was no statistically significant difference in estimated mean daily salt intake between before and after the ARB treatment.

Histological Changes with ARB Treatment

The results of the global sclerosis index obtained by the two nephrologists were identical, and the determinations of whether or not the mesangial matrix expansion ratio and interstitial fibrosis ratio were improved by ARB treatment in each patient were also consistent between the two nephrologists. Although the global glomerular sclerosis ratio was not significantly altered by ARB treatment (from 6.3±8.5% to 10.7±16.1%, $p=0.33$), the mesangial proliferation expansion ratio (from 33.1±10.8% to 22.7±7.8%, $p=0.001$) and the interstitial fibrosis expansion ratio (from 19.9±5.8% to 13.8±4.4%, $p=0.034$) were significantly reduced (Table 3 and Fig. 1). ARB therapy significantly improved these histological findings, *i.e.*, the mesangial expansion ratio and interstitial fibrosis ratio, in both IgA-GN and non–IgA-GN patients.

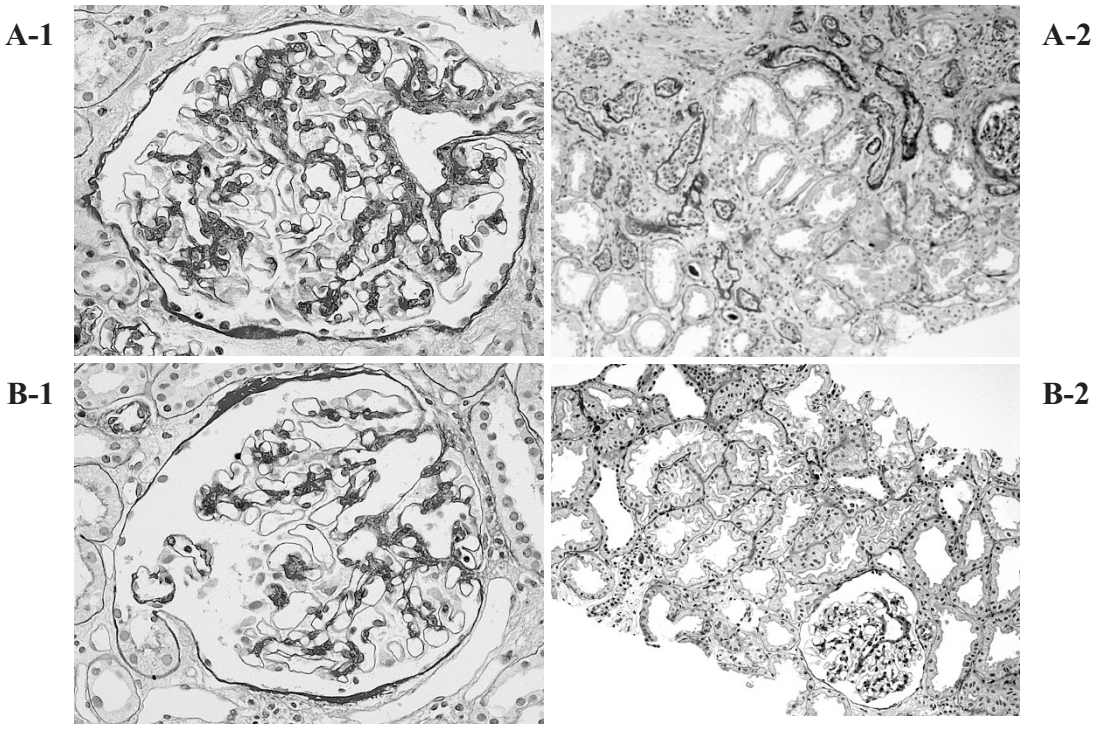

Fig. 1. *Renal pathological findings at first (A) and second (B) biopsy (PAS-staining). A-1: Moderate expansion of the glomerular mesangial matrix. B-1: Glomerular changes in the second biopsy specimen in the same patient as in A-1 (original magnification: ×400). Note the significant reduction in mesangial matrix expansion after ARB treatment. Interstitial fibrosis (A-2, B-2) has also showed significant regression by ARB treatment. A-2: The tubulo-interstitium at pre-ARB treatment; and B-2: The tubulo-interstitium after ARB treatment in a second biopsy specimen from the same patient as in A-2 (original magnification: ×100).*

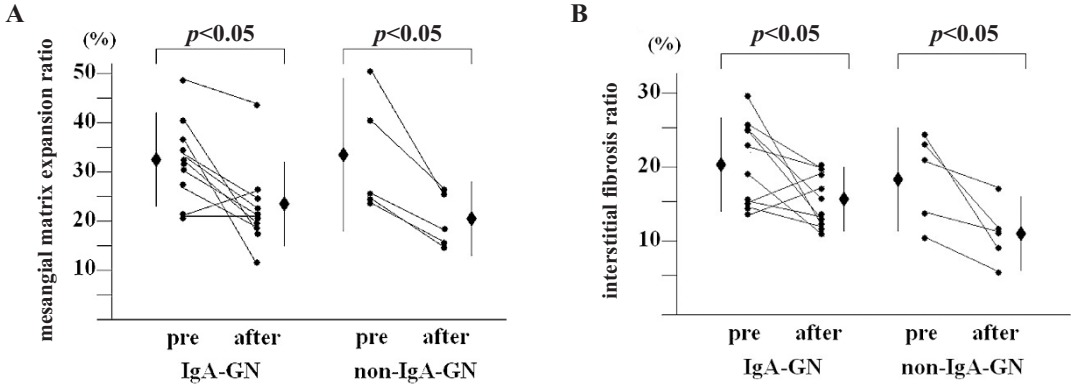

Fig. 2. *Mesangial matrix expansion ratio (A), and interstitial fibrosis ratio (B) of each patient. Pre, pre-treatment values; after, values after ARB treatment.*

Changes of the mesangial matrix expansion ratio and interstitial fibrosis ratio in individual patients are demonstrated in Fig. 2. Eight out of 10 patients with IgA-GN and all 5 patients with non-IgA-GN showed improvements of mesangial matrix expansion and interstitial fibrosis. Two patients with IgA-GN failed to show any improvements of the histological parameters. One was a 64-year-old man and the other was a 47-year-old man. Their initial urinary protein excretion and 24-h Ccr values were 0.30 g/day and 103.8 mL/min/1.73 m^2, and 0.31 g/day and 73.2 mL/min/1.73 m^2, respectively, and their initial

glomerular sclerosis ratios were 6.7% and 20.0%, respectively. However, they clinically responded well to ARB treatment, *i.e.*, the urinary protein excretion decreased (from 0.30 g/day to 0.08 g/day, and 0.31 g/day to 0.03 g/day, respectively) and 24-h Ccr improved (from 103.8 mL/min/1.73 m^2 to 127.6 mL/min/1.73 m^2, and from 73.2 mL/min/1.73 m^2 to 81.8 mL/min/1.73 m^2, respectively) after ARB treatment. Blood pressure control was acceptable (*i.e.*, under 130/80 mmHg) in both patients during the observation period.

Discussion

Our study demonstrated that ARB therapy improved pathological abnormalities, including mesangial matrix expansion and interstitial fibrosis, in patients with mesangial proliferative glomerulonephritis. As far as we know, this is the first evidence that ARBs can improve pathological abnormality in human mesangial proliferative glomerulonephritis. These pathological improvements due to ARB therapy were also accompanied by significant reductions of blood pressure and proteinuria.

Ten of our subjects had IgA nephropathy, which involves immune-complex mediated renal injury. ARB monotherapy achieved pathological improvements in 8 out of these 10 patients with immune-mediated renal injury. Because the serum IgA levels did not change in these patients, non-immune mechanisms, rather than immune-mediated mechanisms, involved in IgA-GN may be the target of ARB therapy. Two patients with IgA-GN did not show regression of renal abnormalities. All of the clinical parameters in these two patients, including proteinuria, microhematuria, 24-h Ccr, and blood pressure, were improved by ARB monotherapy. The glomerular sclerosis ratio also was not worsened by ARB monotherapy in these two patients. Therefore, the reason why pathological regression was not seen in these two patients may have been the short duration of ARB treatment. Although the mean duration of ARB treatment was 28.1 months in this study, it was only 14 and 16 months in these two patients, so pathological regression may require a longer treatment period.

The mechanism by which ARB achieves pathological improvement of renal injury is thought to be due to blockade of angiotensin II activity *via* the angiotensin type 1 receptor. The diverse effects of angiotensin II on the kidney include 1) promotion of glomerular capillary hypertension due to efferent arteriole vasoconstriction (*20*, *21*), 2) stimulation of transforming growth factor-β (TGF-β) production by mesangial cells and tubular epithelial cells (*22–24*), 3) stimulation of matrix protein synthesis (*23*), 4) interstitial fibrosis (*25*, *26*), 5) mesangial cell growth (*27*, *28*), 6) changes of tubular epithelial cell phenotype and tubulointerstitial cell kinetics (*29*), 7) cytokine release from renal cells (*30*), and 8) activation of nuclear factor-κB (NF-κB) and increased monocyte chemoattractant protein-1 (MCP-1) gene expression on mesangial cells, leading to macrophage infiltration (*10*, *31*, *32*). These actions of angiotensin II are known to be the common pathway for several types of renal injury.

Whether regression of existing renal lesions, *i.e.*, established renal structural damages, can occur by ARBs is a very important issue, because ARBs have been thought to improve proteinuria through the alteration of glomerular hemodynamics, and not through renal pathological improvements. Although there have been few studies on this matter, evidence for the regression of renal structural changes has recently been reported. Ma *et al*. reported that ARB therapy caused remodeling of glomerular sclerosis in aging rats (*33*). Remuzzi *et al*. also found regression of renal morphological changes by angiotensin II receptor antagonist therapy in a spontaneous overt nephropathy model (*34*). Although not in the case of ARB, Aldigier *et al*. demonstrated regression of existing glomerulosclerosis by inhibition of aldosterone in 5/6 nephrectomized rats (*35*). Direct evidence for the regression of renal injury in humans was reported by Fioretto *et al*. in 1998 (*36*). They found that the changes of diabetic nephropathy were reversed after pancreatic transplantation in diabetic patients, although more than 5 years after transplantation was needed (*36*). The mesangial fractional volume (proportion of the glomerulus occupied by the mesangium) decreased significantly from 0.33 ± 0.07 (baseline) to 0.27 ± 0.02 (at 10 years after transplantation), mostly because of reduction of the mesangial matrix. It seems that pathological improvements occur after a longer period of time. Morphological improvements were found after mean periods of 28.1 months. Therefore, early intervention with ARB therapy may be important to improve histological damage.

Several limitations of this study bear mention. One was the lack of a control group who were not treated by ARBs, which may weaken our finding that ARBs were directly responsible for the regression of pathological changes in human mesangial proliferative glomerulonephritis. However, a follow up of patients with biopsy-proven certain glomerulonephritis without any treatment could not be allowed from an ethical point of view. Our institutional ethical committee did not allow us to have a prospective control study using a non-medicated control group. Other limitations were the wide range for the timing of re-biopsy, the ARB variability, and the small sample size. However, we believe that the findings obtained in our preliminary study will provide new insight into the action of ARBs, *i.e.*, ARBs can regress the pathological damages not only in animal models but also in human mesangial proliferative glomerulonephritis.

In conclusion, although the precise mechanism could not be elucidated, we have demonstrated that ARBs could cause the regression of renal pathological injury in patients with mild-to-moderate mesangial proliferative glomerulonephritis. ARB therapy modulates glomerular hemodynamics by dilation of efferent glomerular arterioles to reduce glomerular capillary hypertension, leading to the reduction of proteinuria. Besides its hemodynamic actions, ARB therapy also improved histological damages, including mesangial matrix

expansion and interstitial fibrosis expansion, in patients with mesangial proliferative glomerulonephritis. ARBs may have antiproliferative and antifibrotic effects *via* inhibition of proliferative and fibrotic cytokines such as TGF-β, as has been shown in experimental animal models of glomerulonephritis. However, the precise mechanisms involved in the regression of renal pathological injury remain to be elucidated.

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